Confirmation and Efficacy Tests Against Codling Moth and Oriental Fruit Moth in Peaches and Nectarines Using Combination Heat and Controlled Atmosphere Treatments

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ABSTRACT Two high-temperature, forced air treatments under controlled atmosphere conditions, called CATTS for controlled atmosphere/temperature treatment system, were developed for control of all life stages of codling moth, *Cydia pomonella* (L.), and oriental fruit moth, *Grapholita molesta* (Busck), infesting peaches and nectarines (both *Prunus* spp.). These treatments were used in efficacy and confirmation tests to kill >5,000 fourth instar oriental fruit moths and >30,000 fourth instar codling moths with zero survivors. The treatments consist of linear heating rates of either 12 or 24°C/h to a final chamber temperature under a 1% O_2, 15% CO_2, and >90% RH atmosphere with air speed between 1.2 and 2.0 m/s. At a 12°C linear chamber heating rate, treatment takes ≈3 h to reach a final chamber temperature of 46°C. The average lowest core temperatures of the fruit reached 43.8°C within the last 30 min of the treatment. At a 24°C linear chamber heating rate, it takes ≈2.5 h to reach a final chamber temperature of 46°C. The average lowest core temperatures of the fruit reached 44.6°C for the last 15 min of the treatment. It also was determined that both treatments did not significantly alter the quality parameters that were evaluated to a degree that would have negatively influenced the marketability of the fruit. Positive benefits of treatment included a slower ripening of treated fruit and an inhibition of the loss of juiciness during storage in some cultivars. These treatments may be used to replacement to methyl bromide fumigation for conventional fruit or as a new treatment for organic fruit contingent upon importing country approval.

KEY WORDS codling moth, oriental fruit moth, stone fruit, quarantine, commodity treatment

Codling moth, *Cydia pomonella* (L.), is a pest of quarantine concern to many countries in the Pacific Rim (e.g., Japan and Taiwan) that import U.S.-produced peaches, *Prunus persica* (L.) (Batsch), and nectarines, *Prunus persica* variety *nectarina* (Aiton.) Maxim (NWHC 2006). Oriental fruit moth, *Grapholita molesta* (Busck), is a pest of quarantine concern to Mexico and British Columbia, Canada, two major trading partners in the Western Hemisphere (NWHC 2006). Current control measures used to prevent accidental introduction of these pests to our trading partners are fumigation with methyl bromide (for control of codling moth) (NWHC 2006) and extensive systems approach (for oriental fruit moth to Mexico and British Columbia and codling moth into Taiwan) (NWHC 2006). Fumigation of peaches and nectarines with methyl bromide often results in significant phytotoxicity, sometimes with >50% of the load damaged beyond marketability (G. Van Sickle, personal communication). In addition, the cost of methyl bromide has increased from $5/100 lb (220 kg) tank in 1992 to nearly $1,000 today. The systems approach worked well for the first few years after it was initiated in California, but then additional pests were added to the control list for Mexico, making security difficult to attain (R. Neenan, personal communication). Because of these problems with the quarantine measures, the commercial industry in California and the organic industry in Washington state wanted a nonchemical alternative to achieve export quarantine goals (G. Van Sickle and H. Ostenson, personal communication).

We were testing a new approach for attaining control of internal feeding pests in tree fruits without significantly compromising fruit quality (Neven et al. 2001; Neven 2003a, 2005; Obenland et al. 2005). This approach is called controlled atmosphere temperature treatment system (CATTS) (Neven and Mitcham 1996). Whereas many tropical and subtropical fruits are treated with hot forced air to kill internal pests (Armstrong 1994), CATTS adds a controlled atmosphere (CA) to the heat treatment. The low-oxygen environment impairs the insects' ability to acclimate to the heat treatment, whereas the elevated carbon dioxide levels help shift the internal pH of the insect and maintain spiracle opening, thereby providing additional stress not incurred during a regular heat treatment (Neven 2003b) and reducing total treatment

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time by approximately one-half (Neven and Mitcham 1996, Neven 2005). A shorter treatment time reduces damage caused by excessive heating during longer treatments where CA is not used. CATTS treatments for sweet cherries, apples, pears, peaches, and nectarines resulted in acceptable market quality of the fruit after treatment and normal cold storage (Neven and Mitcham 1996, Neven et al. 2001, Shellie et al. 2001, Obenland et al. 2005). For peaches and nectarines, Obenland et al. (2005) found that although in some cultivars there was a slight amount of surface injury, most cultivars responded well to the treatment, and there was a significant delay in ripening. In addition, trained taste panels were only slightly able to discern a difference between untreated and treated fruit. These differences were minor and thought not to be discernible to the general public.

Two CATTS treatments for peaches and nectarines were developed. One treatment was similar to that developed for codling moth and oriental fruit moth in apples (*Malus* spp.) and used a heating rate of 12°C/h under a 1% O_2, 15% CO_2 atmosphere. The second treatment was developed to follow the core temperatures of peaches and nectarines when the fruit were subjected to a 500°C/h chamber heating rate, which was determined to be ≈24°C/h. Our hypothesis was that if the fruit were not heated beyond their thermal capacity, phytotoxicity would be minimized. Accommodating for thermal capacity of the fruit worked to help minimize phytotoxicity in for apples and stone fruits (Neven et al. 2001, Obenland et al. 2005)

Included in this report are the determinations of the most tolerant infestive stage, most tolerant species, comparative efficacy, and confirmation tests of two CATTS treatments against codling moth and oriental fruit moth in peaches and nectarines.

Materials and Methods

CATTS Chamber. The CATTS chamber (Techni-Systems, Chelan, WA) used in these studies was described previously (Neven and Mitcham 1996). Treatment boxes used in these tests were standard vented bottom OnoPac (Hilo, HI) papaya treatment boxes (38.1 by 53.3 by 15.2 cm) described previously by Armstrong et al. (1989). The bottom of the boxes was lined with nylon organdy to prevent larvae from dropping out of the box during treatment. Infested fruits were placed into the boxes, and three temperature probes were placed randomly under one surface of a fruit and into two fruit cores. The inner sliding doors of the chamber were closed and sealed with hot glue on the outer edges and duct tape on the inner edges. The outer door was shut and held in place with door clamps. The treatment chamber was purged with nitrogen until the oxygen level reached 2%, and then carbon dioxide injection was initiated, the final atmospheric composition containing 1% oxygen and 15% carbon dioxide by using O_2/CO_2 monitor as described previously (Neven and Mitcham 1996). The heat treatment was then started using a heating rate of either 12 or 24°C/h to a final chamber temperature of 46°C. The dew point was set to 2°C below the fruit surface temperature, and air speed was set at 1.2–2.0 m/s. Dew point and anemometers were described previously (Neven and Mitcham 1996). When time points were taken during a treatment, the box exchanger was attached to the frame of the CATTS chamber. The box exchanger was designed to be attached the front of the CATTS chamber to minimize loss of atmospheric conditions during loading (see description, see Neven 2005). The box exchanger was flushed with nitrogen for 2 min before removing the box from the chamber to help maintain CA levels in the CATTS chamber during exchanges. Exchange of boxes normally took between 8 and 15 s. After the removal of the box from the chamber, the box exchanger was detached from the chamber and the outer door was sealed.

Insects. Both codling moth and oriental fruit moth were reared in the laboratory on codling moth diet (Toba and Howell 1991). There seems to be a discrepancy in the literature on how many instars there are in oriental fruit moth. Tortricid Pests (van der Geest and Evenhuis 1991), p. 393, states that there are four instars of oriental fruit moth. Head capsule widths are 0.19–0.25 mm for the first instar, 0.30–0.44 mm for the second instar, 0.47–0.79 mm for the third instar, and 0.82–1.19 mm for the fourth instar. In addition, "Under certain conditions, including relatively high temperatures (25–30°C), the larvae may pass through an additional fifth instar. The widths of the head capsules of these individuals overlap broadly with those of larvae from cohorts which pass through only four instars, but are distinct from earlier instars within their own (five-instar) cohort (Russell 1986)." We have noted only four instars of oriental fruit moth in our colony. Our colony is reared at constant temperatures and is not exposed to heat stress. Therefore, when the fourth instar of oriental fruit moth is referenced, it is in respect to the last larval stadium.

All insects were reared at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h. Females were allowed to oviposit in wax-coated oviposition bags (9 by 15 by 27.5 cm, depth by width by length) containing 250 pairs (500 total) of adults for 24–48 h at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h. The bag was placed at 2°C for 5 min to facilitate removal of moths. The bags were held at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h until first instars hatched. First instars were collected from oviposition bags. Larvae from the second–fifth instar for codling moth and second–fourth instar for oriental fruit moth were removed from the artificial diet by hand and placed directly onto the fruit used for testing. Tests with eggs were performed by placing 250 2-d-old moths onto 4.54 kg of organically grown nectarines that were placed into a plastic box (8.5 by 27 by 37 cm, depth by width by length). The bottom and top were lined with unbleached muslin to prevent oviposition on the plastic box. Fruit with moths were held for 24 h at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h after which the box was placed at 2°C for 5 min to facilitate removal of moths. The boxes containing fruit with eggs

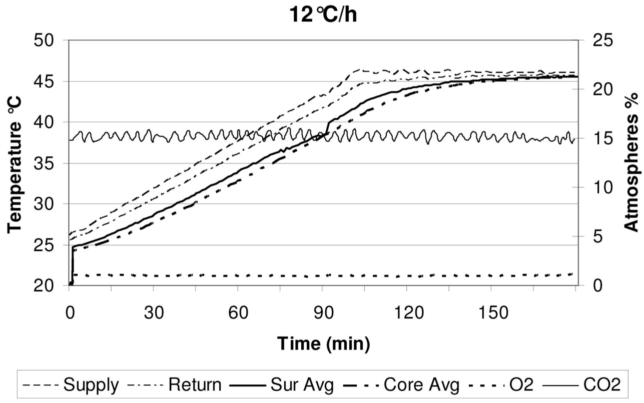

Fig. 1. Treatment profile of 12°C/h CATTS treatment of codling moth in peaches obtained from the 9 September 2003 treatment. Supply air temperature (––), return air temperature (— • —), fruit surface average temperature (▬▬), fruit core average temperature (— • •), % O_2 (– – –), and % CO_2 (——). Atmosphere levels are indicated by right-hand y-axis.

on them were held at rearing conditions until the desired egg stage was reached. The white egg stage for both codling moth and oriental fruit moth was 0–2 d old. The red ring egg stage was 2–3 d for codling moth and oriental fruit moth. The blackhead egg stage was 4–7 d for codling moth and 3–5 d for oriental fruit moth.

Organically grown peaches and nectarines were used as host for infestation with codling moth and oriental fruit moth larvae. The fruit were divided into 6.82-kg lots in plastic storage boxes (41 by 57.5 by 13.2 cm, width by length by depth). In total, 200 larvae of each stage were applied to the 6.82 kg of mature fruit. The top of the box was lined with double stick tape to which a length of nylon organdy was adhered. The top was then sealed with the lid to the storage box. The infested fruit were placed into an environmental controlled room held at 23°C, 50–60% RH, and a photoperiod of 16:8 (L:D) h overnight. A group of 100 larvae of the same stage were used to infest 3.41 kg of fruit to serve as untreated controls for each replicate. Before treatment, infested fruit were removed from the boxes and transferred into the CATTS treatment boxes (OnoPac). Any insects outside of the fruit were counted and subtracted from the total infested number to obtain the total number of insects actually receiving the treatment.

CATTS Treatments. Two separate CATTS treatments were developed for killing codling moth and oriental fruit moth in stone fruits. Initial fruit core temperatures ranged from 18 to 20°C. Core temperatures of the fruit during the 12°C/h treatment reached 43.8°C and remained there for 30 min (Fig. 1; Table 1). Core temperatures of the fruit during the 24°C/h treatment reached 43.5°C and remained there for 15 min (Fig. 2; Table 2). All treated and control infested fruits were subjected to forced air cooling by using a box fan in a 0°C cold room for 15 min and then stored overnight at 0°C to prevent accidental reinfestation of treated fruit. Overnight cold storage of codling moth does not significantly affect mortality (Neven 1994), and all controls received the overnight cold storage treatment that was accounted for in the calculation of corrected mortality.

Most Tolerant Stage. The determination of the most tolerant stage is the first step in developing a physical quarantine treatment. The three egg stages (white, red ring, and blackhead) and instars of both codling moth and oriental fruit moth were subjected to the 12°C/h CATTS treatment. For each stage, 120 larvae or eggs were used per treatment time per replication. There were five treatment times of 0, 1.5, 2.0, 2.5, and 3.0 h, where the 0-h treatment time was used as the untreated control. The treatments were replicated four times. After CATTS treatments, infested fruit were placed at 2°C overnight before evaluation. Fruit were removed from the cold room and allowed to warm to room temperature (20°C) after which fruit

Table 1. Average and minimum core low temperatures (°C) of peaches and nectarines subjected to 12°C/h CATTS treatment

Species	Fruit	No. runs	30-min core low avg ± SEM	30-min core low min.	15-min core low avg ± SEM	15-min core low min.	Core low end temp avg ± SEM	Core low end temp min.
CM	Nectarine	19	44.5 ± 0.06	43.9	44.8 ± 0.09	44.0	44.9 ± 0.11	44.0
CM	Peach	18	44.4 ± 0.05	43.8	44.8 ± 0.06	44.3	45.1 ± 0.07	44.6
CM	Both[a]	37	44.5 ± 0.04	43.8	44.8 ± 0.05	44.0	45.0 ± 0.06	44.0
OFM	Both[a]	4	44.7 ± 0.04	44.6	45.2 ± 0.11	45.0	45.4 ± 0.13	45.2
Both[b]	Both[a]	41	44.5 ± 0.04	43.8	44.8 ± 0.05	44.0	45.0 ± 0.06	44.0

Core low temperature averages and minimums at 30 and 15 min from the end of the treatment and at the end of the treatment for all efficacious treatments are listed. Treatments are listed for tests against codling moth (CM) and oriental fruit moth (OFM).
[a] Data combined for both peach and nectarine tests.
[b] Data combined for both codling moth and oriental fruit moth tests.

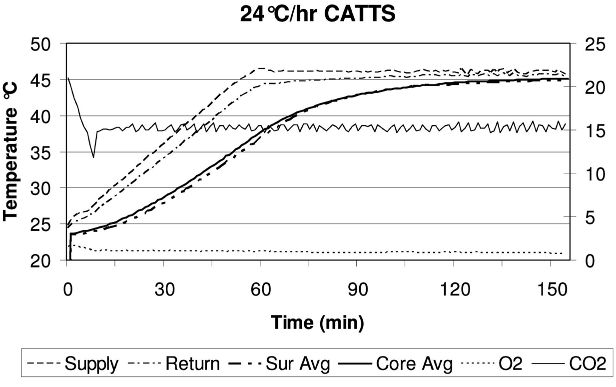

Fig. 2. Treatment profile of 24°C/h CATTS treatment of codling moth in peaches obtained from the 27 August 2003 treatment. Supply air temperature (– –), return air temperature (— • —), fruit surface average temperature (— • •), fruit core average temperature (▬▬), % O_2 (– – –), % CO_2 (——). Atmosphere levels are indicated by right-hand y-axis.

were cut open and examined for surviving and dead larvae. A larva was determined to be dead if it showed no movement, a live larva was able to crawl with prodding, and moribund larvae were able to move but not crawl. Moribund larvae were held on organic apples for 7 d, after which they were assessed for mortality. Eggs were examined for hatch directly after treatment and 7–10 d after treatment, depending on the egg stage treated.

Comparative Thermotolerance. Once the most tolerant stage of each species was determined, we used that stage to determine the most tolerant species. The fourth instars of both codling moth and oriental fruit moth were used in the most tolerant species tests. Fruit were infested, and larvae were evaluated as described above (see Insects). Treatments were suboptimal, in which only chamber temperature and total treatment times were met. Core temperature requirements were not adhered to. Two separate treatments were performed on codling moth and oriental fruit moth. One under regular atmospheres (20% O_2, trace CO_2, and balance N_2) and the other under CA conditions (1% O_2, 15% CO_2). Each treatment was replicated four times.

Efficacy Tests. Efficacy tests were performed on the less thermotolerant species to demonstrate effectiveness of the treatment against that species. The fourth instar of oriental fruit moth was subjected to efficacy tests by using both the 24 and 12°C/h heating rate CATTS treatments. Fruit were infested and larvae were evaluated as described above (see Insects). Efficacy tests usually require treating at least 5,000 of the most tolerant stage with no insects surviving the treatment.

Confirmation Tests. Confirmation tests are typically performed on the most tolerant stage of the most tolerant species to demonstrate the effectiveness of the treatment on a large scale. The fourth instar of codling moth was subjected to confirmation tests by using both the 24 and 12°C/h heating rate CATTS treatments. Fruit were infested and larvae were evaluated as described above (see Insects). One set of confirmation test replicates using the 12°C/h CATTS treatment were performed on half peaches and half nectarines, whereas another set of replicates were performed only on nectarines. Treatments on peaches or nectarines were performed in separate replicates. The 24°C/h CATTS treatment was performed half on peaches and the other half on nectarines. Treatments on peaches or nectarines were performed in separate replicates. Treatments were performed on both peaches and nectarines to demonstrate that the treatments would be efficacious on both types of fruits. Evaluation of the control and treated fruit was performed as described above. Confirmation tests require the treatment of >30,000 of the most tolerant stage of the most tolerant species with no insect surviving the treatment.

Table 2. Average and minimum core low temperatures (°C) of peaches and nectarines subjected to 24°C/h CATTS treatments

Species	Fruit	No. runs	30-min core low avg ± SEM	30-min core low min.	15-min core low avg ± SEM	15-min core low min.	Core low end temp avg ± SEM	Core low end temp min.
CM	Nectarine	17	44.0 ± 0.15	42.4	44.7 ± 0.07	44.3	45.1 ± 0.07	44.5
CM	Peach	12	43.9 ± 0.22	41.9	44.4 ± 0.12	43.5	44.9 ± 0.04	44.5
CM	Both[a]	29	44.0 ± 0.12	41.9	44.6 ± 0.07	43.5	44.9 ± 0.06	44.2
OFM	Both[a]	5	43.9 ± 0.47	42.1	44.9 ± 0.09	44.8	45.3 ± 0.08	45.1
Both[b]	Both[a]	34	44.0 ± 0.12	41.9	44.6 ± 0.06	43.5	45.0 ± 0.06	44.2

Core low temperature averages and minimums at 30 and 15 min from the end of the treatment and at the end of the treatment for all efficacious treatments are listed. Treatments are listed for tests against codling moth (CM) and oriental fruit moth (OFM).

[a] Data combined for both peach and nectarine tests.

[b] Data combined for both codling moth and oriental fruit moth tests.

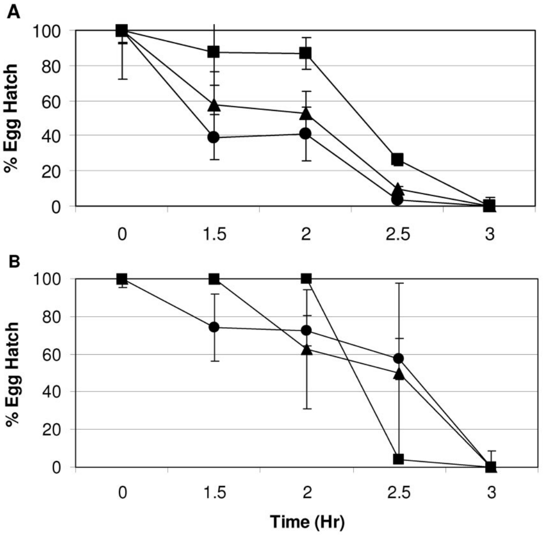

Fig. 3. Percentage of hatch of the egg stages of codling moth (A) and oriental fruit moth (B) in nectarines after a CATTS treatment of 12°C/h to 46°C with a 1% O_2, 15% CO_2 atmosphere. ●, white ring; ▲, red ring; and ■, blackhead.

Treatment Efficacy. Analysis of the average and minimum core fruit temperatures resulting in 100% kill of codling moth and oriental fruit moth were performed to determine requirements defining treatment efficacy. We took the lowest core temperatures at the end of a treatment as well as those 15 and 30 min from the end of the treatment for comparison. Fruit size ranged from 64 to 32 and temperature probes were placed against the pit of the fruit. Only mature, tree ripened organically produced fruit were used in these tests.

Statistics. Control mortality was calculated using Abbott's formula (Abbott 1925). Data were tabulated in Excel 2000 (Microsoft, Redmond, WA) where initial calculations on control mortality and standard error were performed. Probit analysis was performed using PROCPROBIT in SAS version 8.2 (SAS Institute 2000). Time was squared and mortality was not converted. Factorial analysis of variance (ANOVA) was performed on mortality data using SAS where mortality was converted to the arcsine of the square root. Analysis of fruit core temperatures was performed using QuattroPro 10 (Corel Inc. 2001) where averages, minimum, and standard error of the mean were calculated from treatment temperature data obtained from CATTS treatments. Differences between core temperatures were determined using F-test in QuattroPro 10 (Corel Inc. 2001) and ANOVA by using SAS (SAS Institute 2000).

Results

Most Tolerant Stage. The application of a controlled atmosphere to the heat treatment made it difficult to discern differential tolerance among stages of both species (Figs. 3 and 4). When mean mortalities were plotted with SEMs (Figs. 3 and 4), there was signifi-

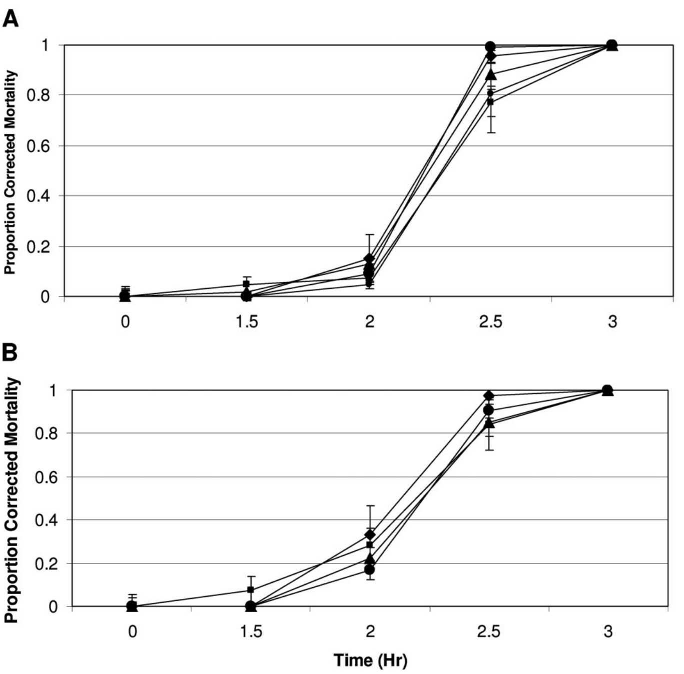

Fig. 4. Proportion corrected mortality of the instars of codling moth (A) and oriental fruit moth (B) in nectarines after a CATTS treatment of 12°C/h to 46°C with a 1% O_2, 15% CO_2 atmosphere. ■, first instar; ●, second instar; ▲, third instar; ♦, fourth instar; and ○, fifth instar.

Table 3. Results of probit analysis of codling moth stages mortality in response to CATTS treatment of 12°C/h heating rate

Stage[a]	LT_{50}	95% CL	95% CL	LT_{90}	95% CL	95% CL	LT_{99}	95% CL	95% CL	df	Intercept[b] χ^2	$Time^{2c}$ χ^2	Intercept[d] Pr > χ^2	$Time^{2e}$ Pr > χ^2
1	1.99	1.59	2.34	2.71	2.40	3.46	3.19	2.78	4.24	8	13.93	17.77	0.0002	<0.0001
2	1.92	1.36	2.33	2.72	2.36	3.64	3.23	2.77	4.54	8	10.25	14.98	0.0014	0.0001
3	1.04	0	1.63	2.49	2.12	3.44	3.22	2.71	4.79	13	0.81	11.6	0.3668*	0.0007
4	2.24	2.08	2.41	2.77	2.59	3.05	3.14	2.90	3.52	18	45.12	45.55	<0.0001	<0.0001
5	2.01	1.74	2.26	2.61	2.37	3.06	3.01	2.70	3.63	14	21.03	25.2	<0.0001	<0.0001
WR	1.34	0.67	1.65	2.28	2.021	2.74	2.82	2.49	3.51	18	6.38	22.96	0.0115	<0.0001
RR	1.36	0	1.85	2.47	2.09	3.42	3.09	2.61	4.54	17	2.55	11.45	0.1102	0.0007
BH	1.91	1.91	0.42	3.27	0.56	2.44	4.06	0.76	3.49	13	4.1	7.96	0.429*	0.0048

* Not significant.
[a] First–fifth Instars are indicated by numerals 1–5. Egg stages are indicated by white ring (WR), red ring (RR), and blackhead (BH).
[b] Chi-square of the intercept.
[c] Chi-square of time squared.
[d] Probability of the intercept.
[e] Probability of the time squared.

cant overlap of the SEMs. When comparing the median lethal time $(LT)_{50}$ values of the instars (Table 3; Fig. 5A) the fourth instar of codling moth was more tolerant than the other instars. Factorial ANOVA followed by Duncan's means separation of the mortality of codling moth larvae in nectarines indicated that the first and fifth instars were less tolerant than the second–fourth instars ($F_{4,\ 66} = 9.53$; $P < 0.0001$), which were equal in tolerance. There were no differences in the tolerance of the egg stages ($F_{2,\ 40} = 0.84$; $P = 0.44$). There was no significant interaction between stage and treatment time for either larvae or eggs ($F_{16,\ 66} = 1.59$; $P = 0.097$; and $F_{8,\ 40} = 1.67$; $P = 0.1365$).

When comparing the LT_{50} values of oriental fruit moth, the fourth instar was slightly more tolerant than the other instars (Table 4; Fig. 5B). Factorial ANOVA of the mortality of oriental fruit moth larvae in nectarines indicated that there were no differences in tolerance in relation to stage ($F_{3,\ 49} = 2.09$; $P = 0.1131$). However, there were significant differences in egg stage tolerance with the blackhead stage being more tolerant than the red ring or the white ring stages ($F_{2,\ 10} = 6.24l$; $P < 0.0174$) based on Duncan's means separation. There was no significant interaction between stage and time for either larvae or eggs ($F_{12,\ 49} = 1.43$; $P = 0.1846$; $F_{8,\ 10} = 0.61$; $P = 0.754$).

Comparison of the LT_{50} plots for codling and oriental fruit moths indicated that the fourth instar of codling moth was more tolerant than the fourth instar of oriental fruit moth (Fig. 5C). When factorial ANOVAs were performed, the interaction between stage and time was significant ($F_{16,\ 116} = 1.83$; $P = 0.0348$) when the mortalities of the larval stages of both species were compared with one another. One-way ANOVA indicated that time was significant for all stages and that the only the zero and 1.5-h treatment time were significantly different ($F_{4,\ 27} = 4.33$; $P = 0.0079$; $F_{27,\ 4} = 9.35$; $P < 0.0001$). Core temperatures at these early treatment times do not reach temperatures where significant larval mortality would occur (Neven and Rehfield 1995) and should not be considered when determining thermotolerance. When ANOVA was run without these time points, there was no difference between the two species ($F_{1,\ 70} = 2.90$; $P = 0.0930$), but stage was significant ($F_{4,\ 70} = 3.35$; $P = 0.0144$) with the fourth instar being the most tolerant based on Duncan's means separation. None of the other interactions were significant.

Probits of the egg stages cannot accurately be compared with those of the instars because the response variable was hatch for eggs and mortality for larvae. In addition, the blackhead for each species resulted in

Table 4. Results of probit analysis of oriental fruit moth stages mortality in response to CATTS treatment of 12°C/h heating rate

Stage[a]	LT_{50}	95% CL	95% CL	LT_{90}	95% CL	95% CL	LT_{99}	95% CL	95% CL	df	Intercept[b] χ^2	$Time^{2c}$ χ^2	Intercept[d] Pr > χ^2	$Time^{2e}$ Pr > χ^2
1	1.96	1.64	2.07	2.67	2.47	3.01	3.13	2.86	3.60	13	32.3	42.25	<0.0001	<0.0001
2	1.89	1.67	2.10	2.68	2.46	3.02	3.18	2.89	3.64	18	31.34	44.33	<0.0001	<0.0001
3	2.15	1.88	2.58	2.60	2.29	3.19	2.91	2.55	3.63	13	37.71	24.05	<0.0001	<0.0001
4	2.02	1.84	2.21	2.50	2.31	2.82	2.82	2.59	3.27	18	27.46	29.73	<0.0001	<0.0001
WR	2.29	1.95	2.66	3.28	2.90	4.13	3.90	3.39	5.09	8	20.15	21.31	<0.0001	<0.0001
RR	2.31	1.551	3.27	2.86	2.45	5.52	3.24	2.74	6.92	8	6.85	7.04	0.0089	0.008
BH	2.18	NE[f]	NE	2.76	NE	NE	3.15	NE	NE	8	3.72	3.82	0.0539*	0.0506

* Not significant.
[a] First–fifth Instars are indicated by numerals 1–5. Egg stages are indicated by white ring (WR), red ring (RR), and black head (BH).
[b] Chi-square of the intercept.
[c] Chi-square of time squared.
[d] Probability of the intercept.
[e] Probability of the time squared.
[f] NE, not estimated.

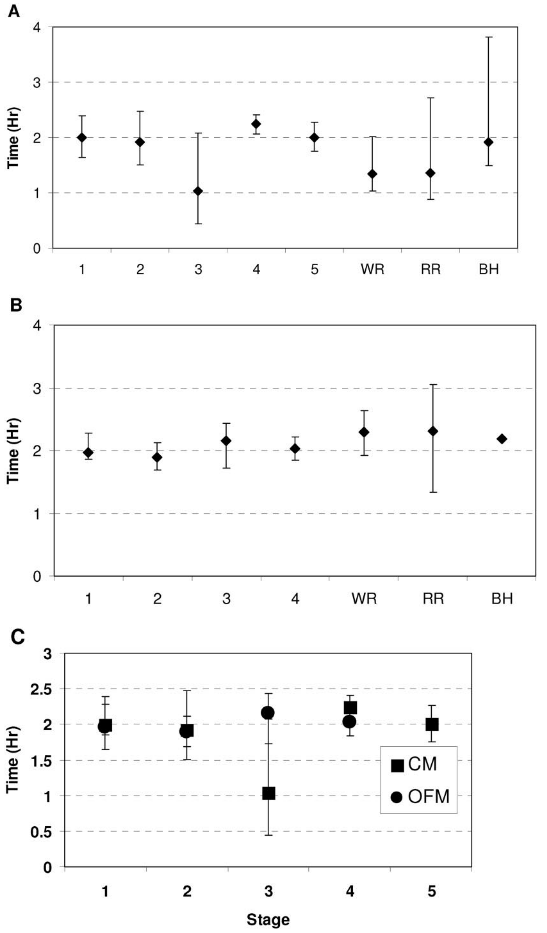

Fig. 5. Calculated LT_{50} values of codling moth (A), and oriental fruit moth (B) stages, and the larvae of codling moth and oriental fruit moth plotted together (C) after 12°C/h CATTS. First–fifth instars are indicated by numerals 1–5. Egg stages are indicated by white ring (WR), red ring (RR), and blackhead (BH).

nonsignificant probabilities of the chi square (Tables 3 and 4). However, when the egg stages of both species were compared, there was significant interaction between stage and species ($F_{2,\ 50} = 4.57$; $P = 0.0151$), but there were no significant interactions for treatment time × species ($F_{4,\ 50} = 1.38$; $P = 0.2558$), stage × treatment time ($F_{8,\ 50} = 1.59$; $P = 0.1506$), or species × stage × treatment time ($F_{8,\ 50} = 0.45$; $P = 0.8853$). When one-way ANOVAs were performed followed by Duncan's means separation, it was found that the whitehead stage of oriental fruit moth was more tolerant than codling moth whitehead ($F_{1,\ 28} = 5.44$; $P = 0.0271$) but that the blackhead and red ring stages of both species were not significantly different from one another ($F_{1,\ 19} = 0.71$; $P = 0.4108$; $F_{1,\ 28} = 3.10$; $P = 0.0890$). When egg hatch was compared for both species, factorial ANOVA indicated that codling moth eggs were more tolerant than oriental fruit moth eggs ($F_{1,\ 50} = 7.99$; $P = 0.0068$) based on Duncan's means

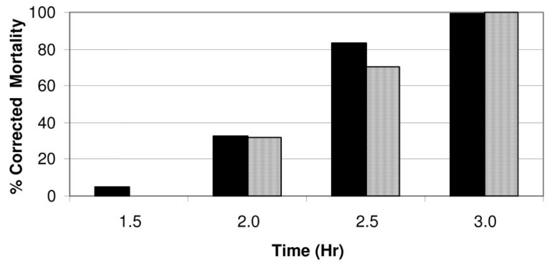

Fig. 6. Percentage of corrected mortality of fourth instar codling moth (■) and oriental fruit moth (▨) to most tolerant species tests by using the12°C/h CATTS treatment (46°C, 1% O_2, 15% CO_2, 3 h). Treatments were suboptimal, in which only chamber temperature and total treatment times were met. Core temperature requirements were not adhered to.

separation. It also indicated that the blackhead stage was more tolerant than both red ring and whitehead stages ($F_{2,\ 50} = 8.98$; $P = 0.0005$), which were both equal in tolerance based on Duncan's means separation.

Previous research (Yokoyama and Miller 1987) determined that the fifth instar of oriental fruit moth was the most thermotolerant stage with the fourth instar being slightly less tolerant. However, because our colony does not produce a fifth instar, the fourth instar of oriental fruit moth would be the most thermotolerant stage. Yokoyama et al. (1991) also determined that the fourth and fifth instars of codling moth were relatively equal in thermotolerance. In addition, comparison of thermotolerance of oriental fruit moth to codling moth (Yokoyama and Miller 1987, Yokoyama et al. 1991, Neven and Rehfield 1995) indicated that codling moth was the more thermotolerant species.

Most Tolerant Species. Most tolerant species data indicate that complete control of oriental fruit moth was achieved in 3 h by using the 12°C/h heating rate CATTS treatment. However, only 99% mortality of the fourth instar codling moth was achieved at the 3-h time point. Complete control of oriental fruit moth was achieved in 2 h by using the 24°C/h CATTS

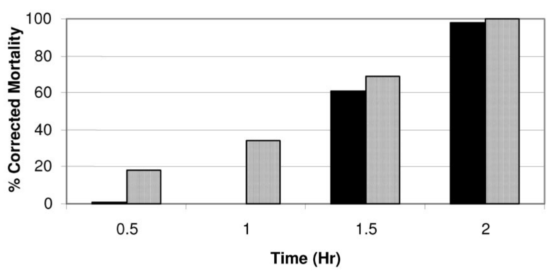

Fig. 7. Percentage of corrected mortality of fourth instar codling moth (■) and oriental fruit moth (▨) to most tolerant species tests by using the 24°C/h CATTS treatment (46°C, 1% O_2, 15% CO_2, 2.0 h). Treatments were suboptimal, in which only chamber temperature and total treatment times were met. Core temperature requirements were not adhered to.

Table 5. Efficacy tests of 12°C/h and 24°C/h CATTS (1% O_2, 15% CO_2, final chamber temperature of 46°C) against fourth instar oriental fruit moth

Treatment	Control	% control mortality	No. treated in fruit	% corrected mortality
12°C/h	900	0.0	5,674	100
24°C/h	901	0.2	5,453	100

Table 7. Confirmation test of 12°C/h CATTS at 1% O_2, 15% CO_2, to a final chamber temperature of 46°C against fourth instar codling moth in nectarines only

Treatment	Control	% control mortality	No. treated in fruit	% corrected mortality
12°C/h	3,601	1.2	30,944	100

treatment (Figs. 6 and 7), but only 99% of codling moth mortality was obtained. Treatments were suboptimal, in which only chamber temperature and total treatment times were met and the requirements for minimal core temperatures were not adhered to, allowing for potential survivors at the last time point. From these tests, it seems that codling moth is slightly more resistant to the CATTS treatments than oriental fruit moth.

Efficacy Tests. Average control mortality was <1% in all treatments. The number killed was adjusted from the control mortality and larval recovery (insects outside of fruit) during transfer from infesting bins to treatment boxes. In total, 5,674 fourth instars of oriental fruit moth were killed with zero survivors in the 12°C/h CATTS treatment, whereas 5,453 fourth instar oriental fruit moth were killed with the 24°C/h CATTS treatment (Table 5). Both treatments resulted in 100% mortality of oriental fruit moth larvae. There was no difference in treatment efficacy in relation to the type of fruit used, either peach or nectarine.

Confirmation Tests. Average control mortality was 1.2–2.4% for both the 24 and 12°C/h CATTS treatments. The number killed was adjusted from the control mortality and larval recovery (insects outside of fruit) during transfer from infesting bins to treatment boxes. In total, 31,154 and 30,884 fourth instars of codling moth were killed with zero survivors in each of the two CATTS treatments (Table 6) by using half peaches and half nectarines. In total, 30,944 fourth instar codling moth were killed with zero survivors by using the 12°C/h CATTS treatment using only nectarines as a host fruit (Table 7). There was no difference in treatment efficacy in relation to the type of fruit used, either peach or nectarine.

Treatment Efficacy. Analysis of the average and minimum core fruit temperatures resulting in 100% kill of codling moth and oriental fruit moth were performed to determine requirements defining treatment efficacy. We took the lowest core temperatures at the end of a treatment as well as those 15 and 30 min from the end of the treatment for comparison. The 12°C/h treatment (Table 1) had minimum requirements for 30 and 15 min from the end as well as the end temperatures of 43.8, 44.0, and 44.0°C, respectively. There was a higher variation in core temperatures in the oriental fruit moth treatments, most likely due to the low number of treatments associated with the efficacy tests. When all treatments were pooled, standard errors of the means were between 0.04 and 0.06°C. For the 24°C/h treatment (Table 2), the minimum requirement for 30 and 15 min from the end as well as the end temperatures were 41.9, 43.5, and 44.2°C, respectively. The highest variability seemed to be in the temperatures 30 min from the end of the treatment, with SEMs ranging from 0.47 to 0.12°C. There were no significant differences in core temperatures when peaches and nectarines were compared for the 12°C/h heating rate CATTS treatments ($F = 1.75$, $P < 0.1949$). When core temperatures were compared between the oriental fruit moth and codling moth 12°C/h CATTS treatments, only the 30 min from the end core temperatures were statistically different from one another (0 = 44.7, 44.4°C; df = 3, 15; $F = 13.99375$; $F \leq 0.000128$). However, considering that the sensitivity of the temperature probes are 0.2°C, the significance of this 0.3°C difference is suspect and may be an artifact of the low sample size of the oriental fruit moth treatment. There were no significant differences between the core temperatures of peaches and nectarines in the codling moth and oriental fruit moth 24°C/h CATTS treatments ($F = 0.08$, $P > 0.9715$) 30 min from the end of the treatment. There were statistically significant differences when the 24°C/h CATTS treatments of oriental fruit moth efficacy tests were compared with the codling moth confirmation tests at 15 min from the end (0 = 44.9, 44.6; df = 4, 28; $F = 3.757454$, $F \leq 0.014373$) and the end core low temperatures (0 = 45.3, 44.9; df = 4, 28; $F = 3.459536$; $F \leq 0.020359$). These differences are 0.3 and 0.4°C and again may be an artifact of the accuracy of the temperature probes and also the small sample size of the oriental fruit moth efficacy tests.

Table 6. Confirmation tests of 12°C/h and 24°C/h CATTS (1% O_2, 15% CO_2, final chamber temperature of 46°C) against fourth instar codling moth in both peach and nectarine

Treatment	Control	% control mortality	No. treated in fruit	% corrected mortality
12°C/h	3,447	2.4	31,154	100
24°C/h	3,672	2.1	30,884	100

Discussion

CATTS treatments using a heating rate of either 12 and 24°C/h to a final chamber temperature of 46°C under a 1% O_2, 15% CO_2 atmosphere were sufficient to control the most tolerant stages of codling moth and oriental fruit moth. Although there was a difference in thermotolerance of the egg and larval stages of each species to heat treatments under regular atmospheres (Yokoyama and Miller 1987, Yokoyama et al. 1991), the application of a controlled atmosphere masks this response. Stage-specific thermotolerance was reported to diminish when heat treatments were accompanied by a low oxygen environment in six tortricid pests (Whiting et al. 1995). Although the heating rate and atmosphere concentrations differed from those used in this study, the pattern of diminishing differential stage thermotolerance was similar to our results. The influence of anoxia on insect physiological response to temperature was documented in flesh fly *Sarcophaga crassipalpis* Macquart cold hardiness response (Yocum and Denlinger 1994) in which anoxic conditions blocked the ability of flesh flies to rapidly cold harden. There is also an indication that anoxia can inhibit the formation of heat shock proteins in codling moth (L.G.N., unpublished data). Heat shock proteins were shown to participate in codling moth thermotolerance (Yin et al. 2006), where it was shown that heat-stressed codling moth larvae produced elevated levels of heat shock proteins that was related increased thermotolerance. Thus, the inhibition of the production of heat shock proteins could mask the differences in thermotolerance in different life stages of an insect.

These treatments are an improvement over traditional methyl bromide fumigation in that they extend shelf-life and in many cases result in better fruit quality when compared with traditional methyl bromide-fumigated fruits (Obenland et al. 2005).

The commercial viability of these treatments is still a concern. We have tested CATTS treatments on packed boxes in a 2-ton (8800-kg) commercial CATTS chamber in George, WA, during summers 2004 and 2005. We were able to force heat through domestic commercial boxes in the 2004 runs. However, when export boxes were used in 2005, heat transfer was more problematic due to the location of the ventilation holes on the ends rather than along the sides of the boxes. We are currently working with the industry to construct a special commercial CATTS unit, which could treat a pallet of boxed fruit. We expect this chamber to be completed some time in 2006.

That we did not find significant differences in treatment efficacy between peaches and nectarines indicates that this treatment is equally effective for both types of fruit. The reason for this similarity may be related to the slow rate of heating used in this system. The treatment of a wide range of fruit sizes by using slow heating allows for equilibrium heating of the fruit, which was based on fruit thermal capacity. Other heat treatments performed on tropical and subtropical fruits do not use this approach; these treatments ramp up the chamber temperature as quickly as possible and hold at the chamber target temperature as the fruit heats up, often with great differentials between chamber and fruit surface temperatures and fruit surface and core temperatures. We found in our work with apples (Neven et al. 1996) that large differences in chamber-to-fruit surface temperatures caused significant phytotoxicity. However, if we took the rate at which the fruit naturally heated and used that linear rate to treat the fruit, phytotoxicity was no longer a problem (Neven et al. 2001, Obenland et al. 2005).

These CATTS treatments were demonstrated to provide complete control of the two major quarantine pests infesting peaches and nectarines. These treatments are efficacious regardless of fruit type (peach or nectarine), and in a flow-through system, regardless of fruit size. These treatments hold great promise as either a replacement for methyl bromide for conventional fruit or as a new direct treatment for organically grown fruit.

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